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Morphologic Alterations of Epidermal Melanocytes and Melanosomes in PUVA Lentigines: A Comparative Ultrastructural Investigation of Lentigines Induced by PUVA and Sunlight*

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Ultrastructural studies were conducted in order to determine morphologic and functional differences in melanocytes and melanosomes in PUVA lentigines and solar lentigines, and light-protected buttock skin. Compared to melanocytes in solar lentigines from 7 subjects and light-protected buttock skin from 5 subjects (none of these subjects had received UV radiation therapy), melanocytes in PUVA lentigines from 6 subjects generally had longer and more numerous dendrites, and showed more active melanogenesis. Basal keratinocytes in PUVA lentigines had a significantly increased frequency of large, single melanosomes, and revealed significantly larger individual melanosomes within compound melanosomes. Other findings in some PUVA lentigines included the close apposition of Langerhans cells to melanocytes, and atypical nuclear, cytoplasmic, and melanosomal alterations, including melanosomal pleomorphism and melanin macroglobules. The presence of relatively large and predominantly single melanosomes in basal keratinocytes of PUVA lentigines suggests more active melanogenesis and/or an irreversible somatic alteration. It will be important to determine the clinical course and ultrastructural findings of PUVA lentigines that persist long after PUVA is discontinued.

Circumscribed pigmented macules may appear in patients treated chronically with photochemotherapy (PUVA) [1–9]. Histologically, these PUVA-induced pigmented macules consist of a lentiginous proliferation of functionally active melanocytes, similar to solar lentigines (SL) [1]. In contrast to SL, melanocytes in PUVA lentigines (PL) are relatively hypertrophic, and sometimes cytologically atypical [1]. The following ultrastructural investigation characterizes the morphologic differences of melanocytes and melanosomes among PL, SL, and light-protected buttock skin (LPS).

METHODS

Subjects

Six white adult males (mean age, 45 years; range, 31–73 years) with prominent PL were selected from patients with psoriasis receiving PUVA according to a standard protocol [10] at the Massachusetts General Hospital Photobiology Unit. PL measuring 4–8 mm in greatest diameter (mean 4 mm) were excised from psoriasis-free skin of the buttock (5 cases) or groin (1 case) a mean of 5.6 years (range, 4–6.5 years) after starting PUVA. These 6 patients had received 148–309 PUVA treatments (mean 222) and 1833–4384 joules/cm^2 (mean 3045) before biopsy. PL were alleged to have appeared only after one or more years of continual PUVA treatment.

Although treatment records revealed acute phototoxic reactions (redness and tenderness) on some areas of the body in 4 of 6 subjects, all subjects denied such reactions on the buttocks or groin while receiving PUVA. Hormonal therapy, exposure to arsenic or ionizing radiation, or a personal or family history of melanoma were denied. One subject received methotrexate prior to starting PUVA. Four of the 6 PUVA subjects reported a history of sun-induced "freckling." The historic burning and tanning responses to sunlight according to a standard grading scheme [10] were reported as type II in 1 subject, type III in 3 subjects, and type IV in 2 subjects.

Twelve white adult volunteers without psoriasis (and never having had hormonal or UV radiation therapy) were studied by the same protocol as the PUVA subjects. Seven SL measuring 4–12 mm (mean, 7 mm) were excised from the upper backs of 6 males and 1 female (mean age, 28 years; range 20–43 years). SL were defined as acquired, circumscribed, flat brown spots on sun-exposed skin, consisting of increased numbers of functionally active melanocytes [1]. LPS was excised from 5 additional white subjects (all males) whose mean age was 35 years (range, 30–39 years). Half of the SL, and all specimens of LPS, were excised during the New England summer months. The 5 subjects who donated LPS denied significant buttock sun exposure during the previous 12 months. The historic burning and tanning responses to sunlight in these 12 subjects were assessed as type I in 2 cases, type II in 5 cases, and type III in 6 cases.

All subjects were studied at the Pigmented Lesion Clinic of Massachusetts General Hospital according to consent guidelines approved by the Subcommittee for Human Studies.

Tissue Preparation

Deep tangential excisions were obtained using 1% lidocaine anesthesia injected around each biopsy site. Pigmented and adjacent normal skin of all 6 PL, all 7 SL, and the 5 specimens of LPS were processed for routine paraffin sections, L-dihydroxyphenylalanine (dopa) paraffin sections, and split dopa preparations. Results of these studies are published elsewhere [1]. Additional sections of these specimens were fixed at room temperature for 2 h in half-strength Karnovsky's solution diluted with 0.2 M cacodylate buffer (pH 7.4), and postfixed at 4°C for 2 h with 2% osmium tetroxide in the same buffer. After dehydration in a graded ethanol series, specimens were passed through propylene oxide and embedded in Epon 812. Ultrathin sections were cut with diamond knives on a Porter Blum MT-2 microtome, counterstained with uranyl acetate and lead citrate solutions, and examined with a Zeiss EM 109 electron microscope operated at 80 kV.

Electron micrographs of basal keratinocytes of each specimen were taken randomly at 12,200× magnification. Prints were enlarged an additional 3× magnification in order to evaluate the distribution pattern and size of melanosomes. The distribution pattern of single melanosomes (SiM) (designated % SiM) was defined as the ratio of SiM to the total number of SiM plus compound melanosomes (CoM) counted, times 100%. At least 1000 melanosomes (SiM plus CoM) were counted in each of the 6 PL and 7 SL. The scarcity of melanosomes in LPS and normal skin adjacent to PL and SL permitted counts of only 100–200 melanosomes for each specimen. Adjacent normal skin was available for ultrastructural studies in only 4 of 6 PL and 2 of 7 SL.

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Abbreviations:
CoM: compound melanosomes
dopa: L-dihydroxyphenylalanine
LPS: light-protected buttock skin
MMG: melanin macroglobules
PL: PUVA lentigines
PUVA: photochemotherapy with oral psoralen and ultraviolet A
SiM: single melanosomes
SL: solar lentigines

In order to compare melanosome size, the largest melanosomes (determined from the 60 largest SiM, or the largest individual melanosome in 60 CoM) were measured in 10 randomly selected prints for each specimen. Only 10–20 SiM per case for SL and LPS, and only 20 CoM per case for LPS, were available for these measurements.

Statistical Analysis

All comparisons were made using the one-sided Fisher's randomization test [11].

RESULTS

Ultrastructural Features

Solar Lentigines (SL): Melanocytes in all SL were increased in number, especially at the sides and tips of epidermal rete ridges. Some melanocytes projected into the dermis, but were still above the basement membrane.

Melanocytes in the vicinity of heavily pigmented basal keratinocytes generally showed evidence of increased activity manifested by increased numbers of melanosomes, enlarged perikarya with well-developed rough endoplasmic reticula, numerous mitochondria, and hypertrophic Golgi complexes (Fig 1). Melanocytic dendrites in SL extended to two (rarely three) levels above the epidermal basal unit. These active melanocytes generally had irregularly shaped nuclei with occasionally prominent nucleoli, but pseudo-inclusion bodies were absent.

Double-nucleated melanocytes were noted in only 1 of the 7 SL. This 1 case also demonstrated focal aggregations of active melanocytes, and autophagocytosis of melanosomes (manifested by the presence of numerous large CoM in melanocytes). The loosely aggregated melanocytes in this 1 case did not show abnormal nuclear contours or degenerative cytoplasmic alterations. Except for *focal* atypical melanosomal autophagocytosis in this 1 case, melanosomal architecture in melanocytes of SL was relatively similar. Most melanosomes were singly disposed, of normal size and shape, and undergoing normal melanization [12]. In some melanocytes of SL, small numbers of melanosomes were spherical and granular instead of ellipsoidal; and small CoM (CoM containing fewer than 4–6 melanosomes per complex), instead of the expected singly disposed melanosomes, were observed focally.

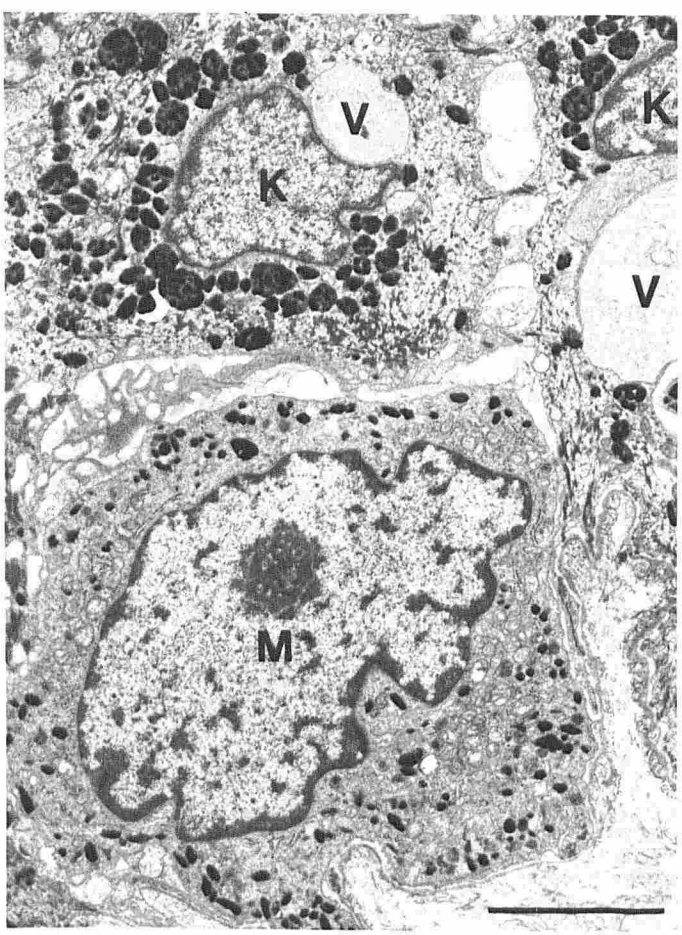


FIG 1. Solar lentigo. Metabolically active melanocyte (*M*), characterized by numerous melanosomes and mitchondria, well-developed rough endoplasmic reticula, and an irregularly shaped nucleus with a prominent nucleolus. Note presence of melanosome complexes and vacuoles (*V*) in keratinocytes (*K*). *Bar* = 1 μm.

Melanocytes in less pigmented zones of SL usually showed evidence of less activity, i.e., fewer well-developed rough endoplasmic reticula, Golgi complexes, and free ribosomes. These relatively "inactive" melanocytes had poorly developed dendrites, revealed numerous bundles of intermediate (100 Å) microfilaments in the perinuclear zone, contained fewer and smaller singly disposed melanosomes, and did not show melanosomal autophagocytosis.

PUVA Lentigines (PL): Compared to adjacent normal skin, melanocytes in PL were increased in number, and frequently aggregated in groups of 2 or 3. Melanocytes were situated focally above the basal epidermal unit in 4 of 6 cases. The vast majority of melanocytes in PL contained well-developed cytoplasmic organelles, occasional lipid droplets, and a greater number of melanosomes than in adjacent normal skin. Compared to LPS, melanocytic dendrites in PL were in general more numerous, longer, and wider, and frequently extended to the third suprabasal layer. In 3 of 6 PL, melanocytic dendrites extended to the fourth suprabasal layer.

Melanocytic nuclei of PL revealed sharply invaginated contours and occasionally large, open nucleoli. Small pseudo-inclusion bodies were noted in 2 of 6 cases (Fig 2*A*). In general, heterochromatin was evenly dispersed, or slightly clumped along the inner leaflet of the nuclear membrane. Double-nucleated melanocytes were observed in 2 PL (Fig 2*B*).

In melanocytes of PL, melanosomes were increased in size and number, usually had a normal configuration (ellipsoidal), and were mostly in developmental stages III and IV. Occasionally, granular melanosomes were observed. Instead of SiM, which were the dominant melanosomal form in melanocytes of LPS, CoM were frequently observed in melanocytes of PL, indicative of melanosomal autophagocytosis (Fig 3). The number of individual melanosomes in these CoM varied, but in general were markedly increased when compared to CoM in melanocytes of SL. Some of the CoM in melanocytes of PL contained up to 100 individual melanosomes.

In melanocytes of 2 PL, ellipsoidal or spherical *melanin macroglobules* (MMG) were noted in small numbers (Fig 4). These MMG were as large as 2 μm in diameter, consisting of amorphous electron-dense material and electron-lucent microvesicles. Each microvesicle measured about 400 Å in diameter. A delimiting membrane surrounded MMG, but was often difficult to appreciate in all photographs.

One of the 6 PL showed striking melanosomal alterations in melanocytes.† Melanosomes revealed marked pleomorphism, with normal (ellipsoidal) and distorted shapes (including granular, crescent, and abortive forms) (Fig 5*A*). Endoplasmic reticula were markedly dilated, and mitochondria were swollen (Fig 5*B*). Mitochondrial swelling was sometimes accompanied by a loss of cisternae and transformation into large vacuoles. Other changes in this one case included autophagic vacuoles and cytoplasmic vacuolization. Focal accumulations of cellular debris in intercellular spaces of keratinocytes suggested degeneration of melanocytes.

The number of Langerhans cells appeared normal in 4 of 6 PL, and decreased in two. In 1 PL, Langerhans cells were noted

† This lesion corresponds to Fig 1H in [1].

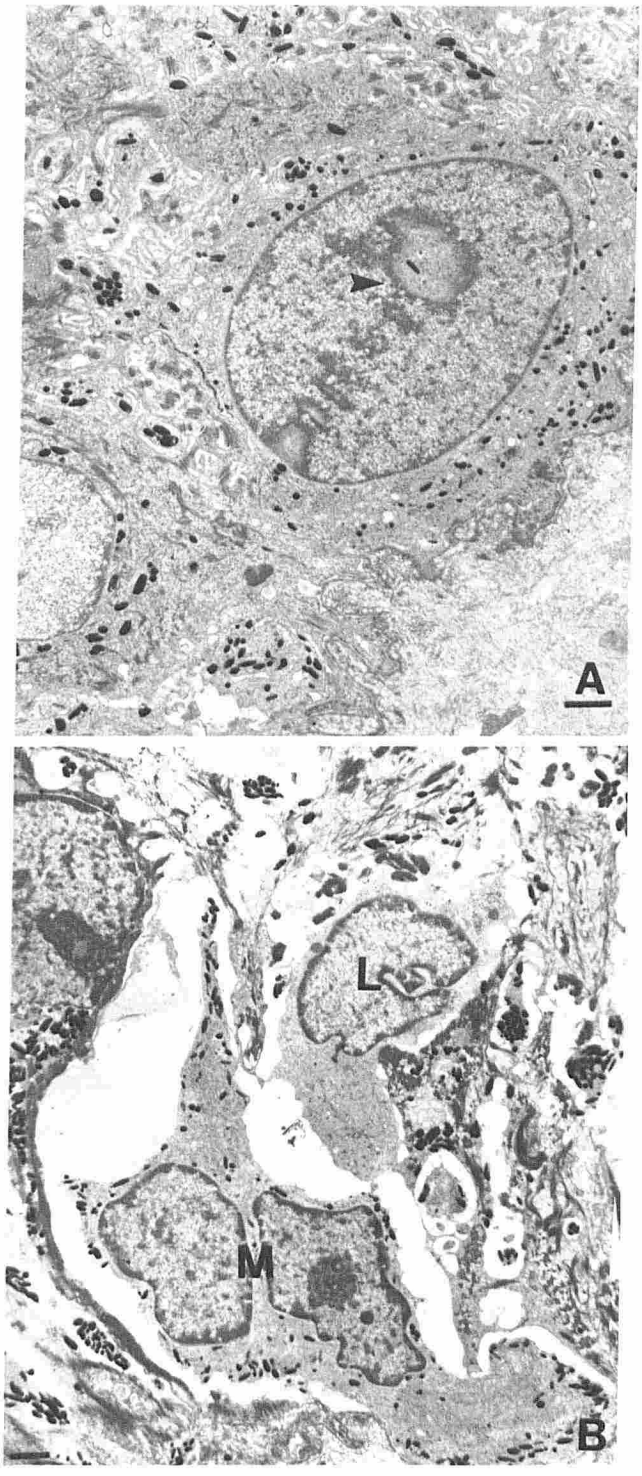


FIG 2. PUVA lentigo. *A*, Pseudo-inclusion with cytoplasmic melanosome (*arrowhead*) in the nucleus of melanocyte (× 5,500). *B*, Close apposition of Langerhans cell (*L*) to double-nucleated melanocyte (*M*). *Bar* = 1 μm.

in close apposition to markedly active melanocytes (Figs 2*B*, 3).

Quantitative Assessment of Melanosome Distribution Pattern and Size

Melanosome Distribution Pattern: A mixture of SiM and CoM was observed in basal keratinocytes of SL, PL, normal skin adjacent to SL and PL, and LPS. Among the 5 specimens of LPS, there was little variation in the proportion of melanosomes that were SiM (range, 14–18%; mean 16%). In contrast, there was marked variation in melanosomal pattern, as well as the total number of melanosomes from cell to cell, in basal keratinocytes of PL, SL, and normal skin adjacent to PL and SL. Occasionally, extraordinarily large CoM were present in keratinocytes of PL (Fig 6). These large CoM were not included in the quantitative studies. Paired melanosomes ("doublets") closely packed in basal keratinocytes were observed occasionally in PL, but were noted only rarely or not at all in SL and LPS.

The mean percent of SiM was on average 3 times greater in PL or in normal skin adjacent to PL than in SL or LPS (Table I).

Melanosome Size: Compared to the typical pattern of CoM (composed of small individual melanosomes) in basal keratinocytes of SL and LPS, melanosomes in basal keratinocytes of PL were unusually large and in the form of SiM. Also, SiM in basal keratinocytes of PL were larger than SiM in basal keratinocytes of SL and LPS (Fig 7*A*,*B*).

In the quantitative assessment, the average mean length and width of SiM in basal keratinocytes were each 2 times greater in PL than in LPS. The average mean length of SiM in basal keratinocytes of PL was 1.5 times greater than in SL. There was no significant difference between the average mean length of SiM in basal keratinocytes of PL compared to normal skin adjacent to PL. The average mean length and width of individual melanosomes in CoM were also significantly larger in basal keratinocytes of PL and normal skin adjacent to PL than in LPS. The average mean length of individual melanosomes (but not the width) in CoM was significantly larger in basal keratinocytes of PL than in SL (Table II).

DISCUSSION

Morphologic similarities, as well as striking differences, characterized our ultrastructural comparisons of melanocytes and melanosomes in PL and SL. Our observations of SL were consistent with previously published findings [12,13], namely, the presence of epidermal hypertrophy and a budding conformation of epidermal rete ridges, and proliferation of functionally active melanocytes. Melanocytes in SL showed variable cytologic changes, directly related to melanogenesis and metabolic activity. Metabolically *inactive* melanocytes in SL were similar to melanocytes in sun-protected skin [14]. The vast majority of melanosomes in melanocytes of SL demonstrated normal melanization and transfer. Although cytoplasmic changes were variable from one melanocyte to another, we detected no bizarre nuclear contours, pleomorphism of melanosomes, or atypical cytologic alterations in SL.

Like SL, PL also revealed an increased number of functionally active melanocytes, consistent with previous quantitative studies using the dopa-paraffin technique [1]. In contrast to SL, however, melanocytes in PL were noted to have highly irregular nuclear contours, occasionally prominent nucleoli and double-nucleated melanocytes, and melanocytic dendrites that were more numerous and longer, often reaching the fourth suprabasal layer. Kanerva et al [4] noted similar findings in PUVA-induced lentigines. Extremely long dendrites have also been noted in melanocytes of PUVA-treated "normal" human skin [15].

The pattern of predominantly *SiM* in basal keratinocytes of lesional and perilesional skin of PL contrasts sharply with the pattern of *CoM* in basal keratinocytes of LPS according to our studies and previously reported observations [16–18]. The melanosomal pattern in basal keratinocytes of SL was more variable than that of either PL or LPS. In general, the distribution pattern of melanosomes in basal keratinocytes is thought to reflect melanosome size, with a tendency for small melanosomes to be complexed, and for large melanosomes to be dispersed singly [16–20]. Konrad and Wolff [18] observed that 80% of melanosomes having average transverse diameters

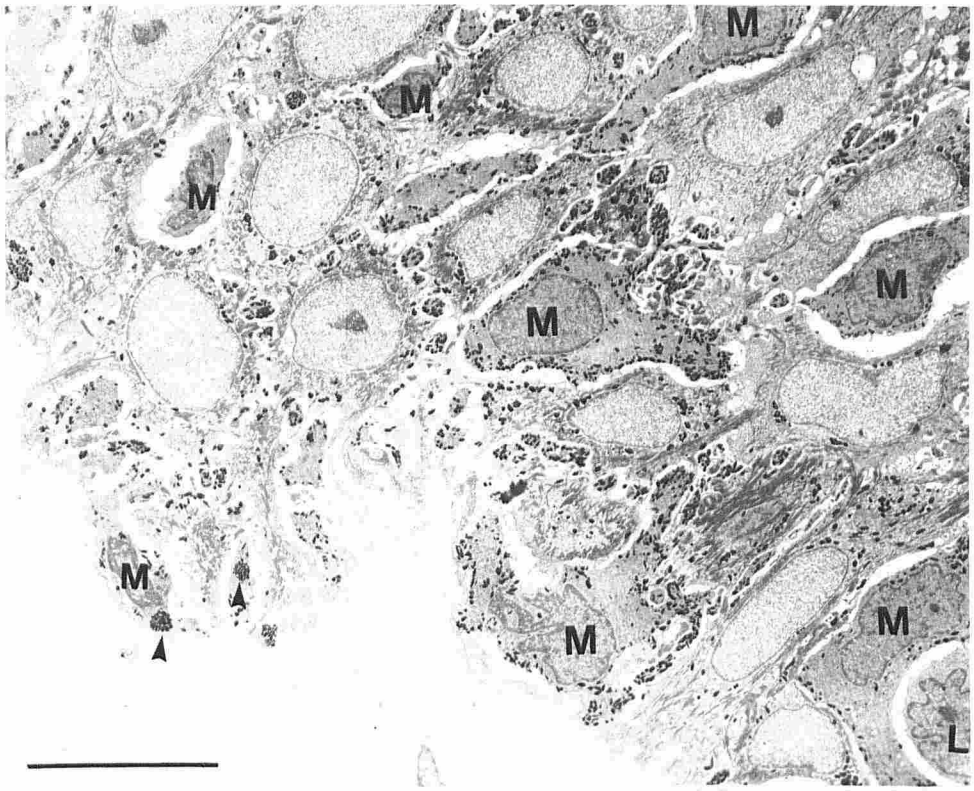


FIG 3. PUVA lentigo. Low-power view showing increased number of active melanocytes (*M*). *Arrowheads* indicate large compound melanosomes. Note close apposition of Langerhans cell (*L*) to melanocyte at the right bottom corner. *Bar* = 10 μm.

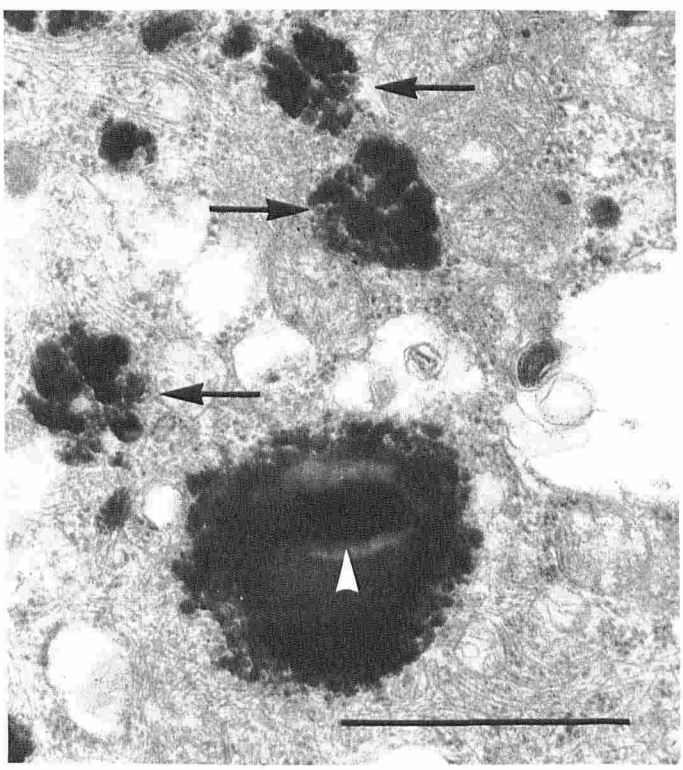


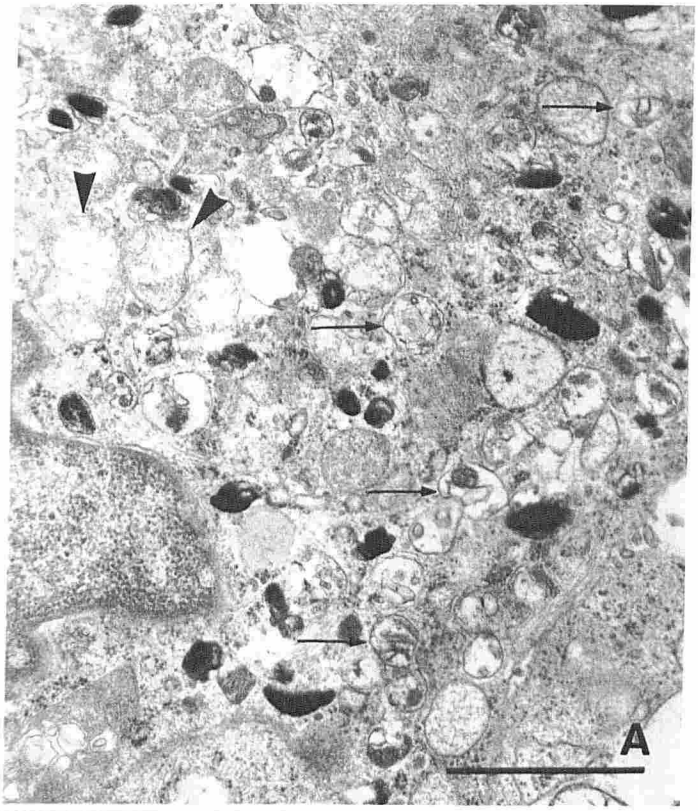
FIG 4. Melanin macroglobule (MMG) in melanocyte of PUVA lentigo. Note simultaneous occurrence of MMG and compound melanosomes (*arrows*). An ellipsoidal melanosome (*arrowhead*) is visible within MMG. *Bar* = 1 μm.

greater than 0.24 μm occurred singly. Olson et al [20] reported the upper limit for the length of melanosomes in complexes to be 0.35 μm, larger melanosomes seen only ocasionally.

Repeated exposures to UVA, UVB, or PUVA have been associated with melanosome enlargement [21]. For basal keratinocytes in Caucasians, Toda et al [17] noted a predominance of large SiM in habitually sun-exposed forearm skin, compared to a predominance of small individual melanosomes in CoM in sun-protected abdominal and buttock skin. Toda et al [17] also noted a change from small melanosomes in complexes to large SiM following topical application of trimethoxypsoralen and a single exposure to long-wave UV radiation in Caucasian skin, and observed that these melanosomal alterations persisted for at least 6 months. Toda et al [17] postulated long-term gene derepression, or the induction of a somatic mutation, to account for these observations. Kanerva et al [4] also noted the presence of singly disposed melanosomes in basal keratinocytes of PUVA-induced lentigines. Basal keratinocytes of melasma have also been observed to contain increased numbers of large SiM [18,22].

Not all investigators agree that PUVA has a significant effect on melanosome size. Pathak et al [21], Hashimoto et al [23], and Zelickson et al [24], observed an increase in melanosome size after repeated exposure to PUVA. In contrast, Zaynoun et al [15] reported no significant change. Kanerva et al [4,5] observed individual variations in melanosome size and distribution patterns in PUVA lentigines. According to our studies, some basal keratinocytes of PL contained mostly CoM, while other keratinocytes within the same specimen contained mostly SiM. The conflicting results among the various studies may be related to biases in sampling, modes of therapy (individual dose and duration of therapy), skin type-related differences in the subjects studied, and heterogeneity of melanosomal pattern within the same specimen.

One of the most notable changes in PL, noted also by Kanerva et al [4], was melanosomal autophagocytosis, defined by the presence of CoM in melanocytes. Melanosomal autophagocytosis may indicate excessive melanosome production and/or a disturbance in melanosome transfer. Autophagocytosis of melanosomes is rarely detected in normal melanocytes. Melanin macroglobules (MMG), similar to structures formerly called macromelanosomes [25,26], were also noted focally in melanocytes of 2 PL. Recent studies of café-au-lait macules in neurofibromatosis strongly support the hypothesis that MMG



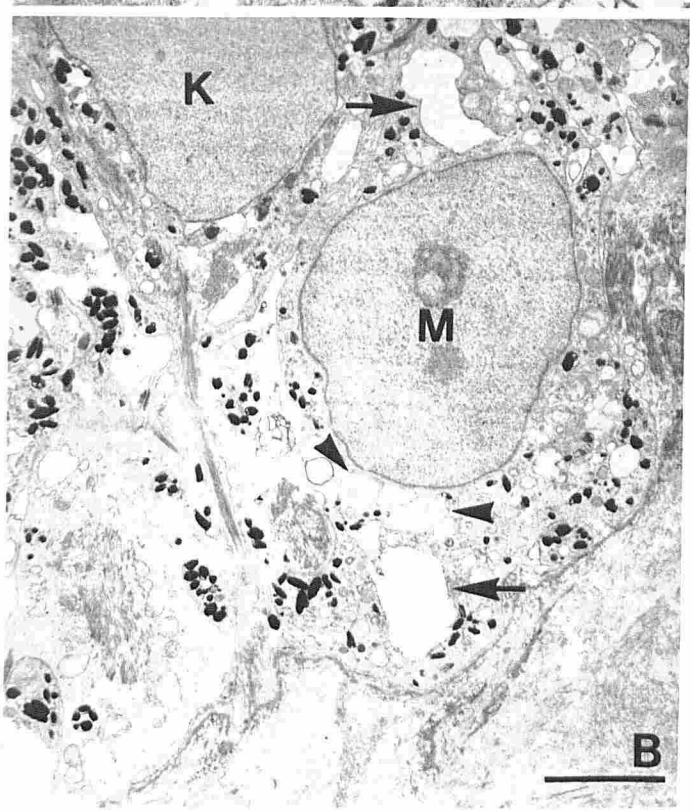


FIG 5. PUVA lentigo, showing melanosomal pleomorphism and degenerative changes. *A*, Abortive melanosomes (*arrows*) and swollen mitchondria (*arrowheads*) are seen. *B*, Degenerative changes in melanocyte, showing dilated cystic endoplasmic reticula (*arrows*) and swollen mitchondria (*arrowheads*). *Bars* = 1 μm.

may be the result of aberrant autophagocytosis (H. Nakagawa and T. B. Fitzpatrick, unpublished observations). A similar phenomenon may be occurring in PL.

Aberrant melanosomes in melanocytes were observed in 1 of

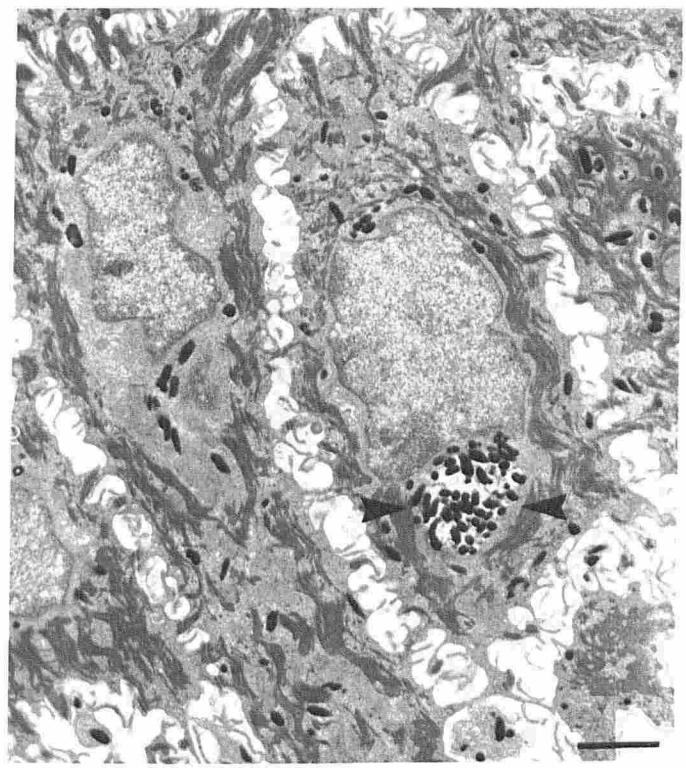


FIG 6. PUVA lentigo, showing huge melanosome complex (*arrowheads*) in basal keratinocyte, associated with large, singly disposed, fully melanized melanosomes. *Bar* = 1 μm.

TABLE I. *Distribution pattern of melanosomes in basal keratinocytes of lesional and adjacent normal skin of PUVA lentigines and solar lentigines, and light-protected buttock skin*

	Average percent of single melanosomes[a] (mean ± SD)
PUVA lentigines	
Lesion[b] (n = 6)	71 ± 15%[c]
ANS[d] (n = 5)	69 ± 14%[c]
Solar lentigines	
Lesion[b] (n = 7)	21 ± 19%
ANS[d] (n = 2)	35 ± 7%
Light-protected	
Buttock skin[d] (n = 5)	16 ± 2%

ANS = adjacent normal skin.

[a] Defined as the ratio of single melanosomes to total melanosomes counted (single melanosomes plus compound melanosomes), × 100%.

[b] 1000–1500 melanosomes counted per case.

[c] $p < 0.005$, when lesional or adjacent normal skin of PUVA lentigines compared to lesional skin of solar lentigines or light-protected buttock skin, respectively.

[d] 100–200 melanosomes counted per case.

our 6 PL. These melanosomes were pleomorphic and demonstrated irregular or incomplete formation of filaments. Pleomorphic melanosomes (especially granular, crescent, and abortive forms) have been observed in cutaneous melanoma [27, 28]. Mishima [29] and Curren et al [30] regard such alterations as highly suspicious for the presence of neoplasia. Mintzis and Silvers [31] and Jakubowitz et al [32] disagree with this notion, because epidermal melanocytes of "benign simulants" may be observed to have similar features. Although the dominant melanosomal shape in the vast majority of our PL was ellipsoidal, the presence of aberrant forms is worthy of further scrutiny, given the documented mutagenic [33] and tumorogenic [34] potential of PUVA.

According to our studies, highly irregular nuclear contours were not present in melanocytes of lesional or perilesional skin of SL, or in LPS. Although highly irregular nuclear contours with large pseudo-inclusion bodies may be characteristic of

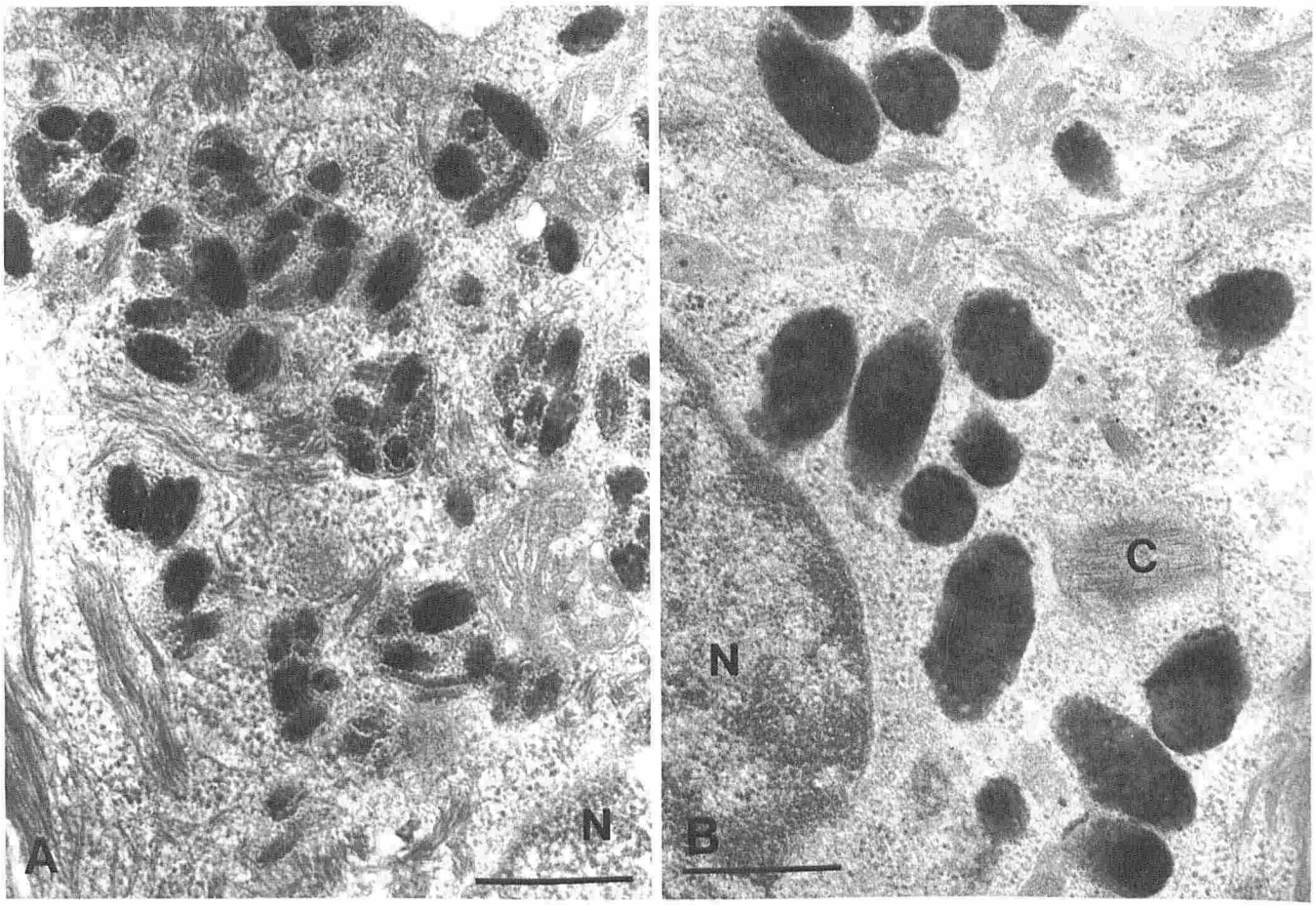


FIG 7. Comparison of melanosomes in keratinocytes of solar lentigo (*A*) and PUVA lentigo (*B*). Note larger, more melanized, and predominantly stage IV melanosomes in (*B*), compared to smaller and less melanized melanosomes in (*A*) (*C* = centriole; *N* = nucleus). *Bars* = 0.5 μm.

TABLE II. *Size comparison of melanosomes in basal keratinocytes of lesional and adjacent normal skin of PUVA lentigines and solar lentigines, and light-protected buttock skin*

Melanosomes	PUVA lentigines		Solar lentigines		Light-protected buttock skin[b] (n = 5)
	Lesion[a] (n = 6)	ANS[b] (n = 4)	Lesion[c] (n = 6)	ANS[b] (n = 2)	
Single melanosomes					
Average mean length, μm	0.62 ± 0.04[d]	0.59 ± 0.04[e]	0.43 ± 0.03	0.42 ± 0.01	0.32 ± 0.02
Average mean width, μm	0.20 ± 0.04[f]	0.21 ± 0.04[e]	0.17 ± 0.02	0.16 ± 0.03	0.12 ± 0.01
Individual melanosomes in compound melanosomes					
Average mean length, μm	0.43 ± 0.01[d]	0.44 ± 0.04[e]	0.33 ± 0.03	0.36 ± 0.01	0.28 ± 0.04
Average mean width, μm	0.15 ± 0.01[f]	0.14 ± 0.01[e]	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.02

ANS = adjacent normal skin.
[a] The 60 largest single melanosomes, or the largest individual melanosomes in 60 compound melanosomes, measured for each case.
[b] The 10 largest single melanosomes, or the largest individual melanosomes in 20 compound melanosomes, measured for each case.
[c] The 20 largest single melanosomes, or the largest individual melanosomes in 60 compound melanosomes, measured for each case.
[d] $p < 0.005$, for comparison of PUVA lentigines with solar lentigines or light-protected buttock skin, respectively.
[e] $p < 0.02$, for comparison of normal skin adjacent to PUVA lentigines with light-protected buttock skin.
[f] $p < 0.005$, for comparison of PUVA lentigines with light-protected buttock skin.

cutaneous melanoma [30], similar findings in PL according to our studies and those by Kanerva et al [4] may indicate a highly active state. PUVA is known to be an effective proliferative stimulus for melanocytes. Double-nucleated melanocytes, noted in 2 of our PL (and 1 SL, but not in LPS), may represent late telophase in this proliferative sequence. Double-nucleated melanocytes and melanocyte clustering have been observed in human skin (pigmented and nonpigmented) treated chronically with PUVA [4,23,24].

Striking mitochondrial swelling was noted in 1 of our PL, accompanied by highly disorganized melanosomes. Abnormal mitochondrial swelling associated with dilated endoplasmic reticula has been observed previously in melanocytes of PUVA-induced lentigines [4] and PUVA-treated "normal" buttock skin [24]. Moreover, this change has been noted for at least 15 months after discontinuing PUVA [24]. In 1 of our PL, we observed the loss of mitochondrial cisternae and the transformation of mitochondria into large vacuoles. These changes, which are a sensitive indication of cellular injury [35], have also been observed in cutaneous melanoma [30].

Striking cytoplasmic, nuclear, and melanosomal alterations have also been observed in melanocytes of nevomelanocytic

nevi in individuals receiving chronic PUVA for psoriasis [36]. However, it is unclear from these studies whether the alterations observed *preceded*, or were the *result* of, PUVA. It is not known whether the melanosomal alterations in melanocytes and keratinocytes of PL, or the abnormal nuclear and cytoplasmic changes in melanocytes of PL, are a reversible effect of UV radiation therapy in general, or an irreversible (and potentially premalignant) alteration. The atypical melanosomal changes we observed in PL (and in normal skin adjacent to PL) have *not* been reported in short-term UVB therapy [14,37]. We are not aware of systematic investigations of pigmentary phenomena induced by therapeutic doses of UVB therapy, nor ultrastructural studies of melanosomes and melanocytes in skin exposed to UVB for treatment periods as long as those experienced by our PUVA subjects. Because the significance and natural course of PL are unknown, it will be important to examine the ultrastructure of PUVA-induced pigmented lesions that persist long after PUVA therapy is discontinued.

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